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Polycationic derivatives of p-tert-butylthiacalix[4]arene in 1,3-alternate stereoisomeric form: New DNA condensing agents

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Abstract

© ISUCT Publishing.One of the important challenges in gene therapy is to search safe and efficient gene carries capable of compacting, protecting, transporting, and delivering nucleic acids into the cell. Most of condensing agents are functionalized on one side with appropriate cationic groups to act as DNA carriers, and, on the other side, with lipid-type chains that permit them to self-aggregate into well-defined aggregation patterns. Calixarenes and their thia-analogues are versatile macrocyclic compounds that can combine the properties of both macrocyclic hosts and self-organizing systems, such as micelles and liposomes. In addition, their relatively easy synthesis, variety of stereoisomeric forms and their low toxicity levels make them really promising vectors in gene delivery applications. It is noteworthy that amphiphilic gene vectors based on calixarenes are generally presented only in cone stereoisomeric form, while 1,3-alternate stereoisomeric form also allows to achieve a spatial separation between hydrophilic and hydrophobic moieties of macrocycle. Herein we report the synthesis of new water-soluble amphiphilic derivatives of p-tert-butylthiacalix[4]-arene in 1,3-alternate stereoisomeric form with different O-alkyl chain length (C4, C6, C14) containing polycationic diethylenetriammonium fragments. The synthesis of new macrocycles was done by stepwise functionalization of parent p-tert-butylthiacalix[4]arene using Mitsunoby reaction and the final introduction of diethylenetriammonium fragments using CuAAC protocol. The structure of new amphiphilic macrocycles was established by 1,2-D NMR and IR spectroscopy, MALDI-TOF spectrometry and elemental analysis. To evaluate their ability to form aggregates the values of critical concentrations of aggregation CCA were determined in aqueous solutions by a well-known fluorimetric method based on the pyrene emission spectra. The CCA values were equal to 24, 25 and 9 μmol for O,O-dibutyl, O,O-dioctyl and O,O-ditetradecyl derivatives, respectively. A significant decrease of CCA value for more lipophilic macrocycle indicates the importance of hydrophobic interactions gor aggregates formation. According to the dynamical and electroforetical light scattering data new macrocycles form stable aggregates with the diameter within 70–100 nm. Such size values indicate that all investigated macrocycles form vesicle-like structures. In the case of O,O-ditetradecyl derivative a significant size decrease up to 50–60 nm was found and can be associated with an increased packing density of the resulting vesicles by enhancing hydrophobic interactions between lipophilic molecule fragments. Also, zeta potential values were measured by electrophoretic light scattering method. Measured values are + 60 – +70 mV, what is in agreement with the polycationic nature of macrocycles, and correspond to the formation of colloidal systems with high stability. Binding of new polycationic macrocycles with nucleic acid was investigated using calf thymus DNA as model biopolymer and well-known

DNA intercalator ethidium bromide. According to the dynamical, electroforetic light scattering data and fluorescent spectroscopy it was found that new polycationic macrocycles effectively interact with calf thymus DNA. Addition of all synthesized macrocycles in concentrations below the CAC does not lead to a significant change in the size of the calf thymus DNA, while at concentrations higher than that of CAC a significant compression of DNA in 2–5 times is observed. Increased lipophilicity of the macrocycles leads to the formation of more compact lipoplex. Furthermore it was found that ethidium bromide removal can be promoted by hydrophobic interactions between the planar aromatic ethidium ring and the surfactant tail groups. The proof of the ethidium bromide migration onto the surfactant tail groups is the non-linear Stern-Volmer curve having pronounced plateau accompanied by a bathochromic shift of the emission maximum at high concentration of o-tetradecyl substituted macrocycle that may be due to the location of the dye in the hydrophobic unit of aggregate.

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Keywords

Calf thymus DNA, Cationic amphiphiles, DNA condensation, Ethidium bromide, P-ter--butylthiacalix[4]arene